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(54) Title: HUMAN INTERLEUKIN VARIANTS GENERATED BY ALTERNATIVE SPLICING

(57) Abstract

Novel splice mutants of interleukins-2 and 4 are disclosed, which contain exons 1, 3 and 4 of the full-length mRNAs, but have exon 2 deleted. The proteins resulting from the expression of these splice mutants are useful in regulating the activity of the full-length interleukins.

growth factors which do not share primary sequence homology. Powers et al (Powers et al. (1992) *Science* 256:1673) speculated that IL-4 contains two binding sites for its receptor, based upon analogy to the growth hormone/growth hormone receptor system (De Vos et al. (1992) *Science* 255:306). The first binding site is predicted to involve IL-4 helices α_A and α_C, whereas the second site is predicted to involve helix α_D, strand B_A, and the connecting loop between strand B_A and helix α_B (Powers et al. (1992) *Science* 256:1673). One predicted IL-4/IL-4 receptor interaction site, Asp^{31}, lies within the strand B_A of exon 2. Exon 2 also contains Cys^{24}, which forms an intramolecular disulfide bond with Cys^{65}. Disruption of this disulfide bond, which would occur in IL-4δ2, is not critical for the biologic activity of mutant IL-4 molecules (Kruse et al. (1991) *FEBS Letters* 286:58).

IL-4 belongs to a multigene family of cytokines that share chromosomal location and molecular organization and structure (Boulay et al. (1992) *J. Biol. Chem.* 267:20525). Members of the family include IL-2, IL-3, IL-4, IL-5, and GM-CSF.

Similar to the IL-4 gene, the IL-2 gene is composed of 4 exons, with exon 2 the shortest at 60 bp (Fujita et al. (1983) *Proc. Natl. Acad. Sci. USA* 80:7437). It has been suggested that the IL-2 molecul has a configuration

of left-handed alpha-helices and B sheets similar to that of IL-4 (Bazan (1992) *Science* 257:410). Exon 2 of IL-2 (amino acid residues 31 to 50) encodes a ß sheet, a short α helix, and the loop connecting helices α_A and α_B (Bazan (1992) *Science* 257:410), a region which is similar to that encoded by exon 2 of IL-4 (Powers et al. (1992) *Science* 256:1673). Exon 2 of IL-2 encodes the portion of the IL-2 molecule that binds the α chain (p55) of the IL-2 receptor (Sauve et al. (1991) *Proc. Natl. Acad. Sci. USA* 88:4636).

IL-4 has been shown to co-stimulate proliferation of resting B cells with anti-IgM antibodies (Howard et al. (1982) *J. Exp. Med.* 155:914), rescue resting B cells from apoptosis (Illera et al. (1993) *J.Immunol.* 151:3521), induce Ig production by activated B cells (Defiance et al. (1988) *J. Immunol.* 141:2000), and regulate isotype switching to IgG_1 and IgE in mice (Coffman et al. (1986) *J. Immunol.* 136:4538) (Vitetta et al. (1985) *J. Exp. Med.* 162:1726), and IgG_4 and IgE in humans (Lundgren et al. (1989) *Eur. J. Immunol.* 13:131). IL-4 exposure has been demonstrated to increase the number of IgM (Shields et al. (1989) *Immunology* 66:224), CD23 (10-12), MHC class II molecules (Rousset et al. (1988) *J. Immunol.* 140:2625) (Roehm et al. (1984) *J. Exp. Med.* 160:679), LFA-1 and LFA-3 (Rousset et al. (1989) *J. Immunol.* 143:1490), and IL-4 receptor (IL-4R) (Renz et al. (1991) *J. Immunol.* 146:3049) molecules on the surface of B cells. In T

cells, IL-4 has been shown to promote proliferation (Fernandez-Botran et al. (1986) *J. Exp. Med.* 164:580) (Mosmann et al. (1986) *Proc. Natl. Acad. Sci. USA* 83:5654) (Mitchell et al. (1989) *J. Immunol.* 142:1548), generation of the Th2 phenotype (Fernandez-Botran et al. (1986) *J. Exp. Med.* 164:580) (Le Gros et al. (1990) *J. Exp. Med.* 172:921) and expression of IL-4R (Renz et al. (1991) *J. Immunol.* 146:3049).

IL-4 exhibits a synergistic effect with IL-3 in promoting the growth of mast cells (Mosmann et al. (1986) *Proc. Natl. Acad. Sci. USA* 83:5654). IL-4 activates macrophages to increase tumoricidal activity, MHC class II expression, and binding of IgG immune complexes (Crawford et al. (1987) *J. Immunol.* 139:135). Precursors of erythroid cells, megakaryocytes, and granulocytes--macrophages can be co-stimulated with IL-4 to increase colony formation (Peschell et al. (1987) *Blood* 70:254). IL-4 also stimulates proliferation (Feghali et al. (1982) *Clin. Immunol. Immunopathol.* 63:182), chemotaxis (Postlethewaite et al. (1991) *J. Clin. Invest.* 87:2147), extracellular matrix production (Postlethewaite et al. (1992) *J. Clin. Invest.* 90:1479), and intercellular adhesion molecule-1 (ICAM-1) expression (Piela-Smith et al. (1992) *J. Immunol.* 148:1375) by fibroblasts.

Interleukin-2 (IL-2) is a T cell growth factor secreted by amplifying T cells (T_A), which stimulate proliferation and differentiation of cytotoxic T cells

(T_c). T_c blast cells express surface receptor for IL-2. The IL-2 receptor (IL-2) is composed of 3 separate proteins p55 (α chain), p75 (β chain), and p65 (δ chain). In different combinations, these chains give rise to various forms of the IL-2R with different affinities and capacity to transduce proliferative signals (Taniguchi et al. (1993) *Cell* 73:5). Similarly, the IL-4R consists of at least two chains. The first IL-4R chain which was described shares significant homology to the β chain of the IL-2R and other members of the growth factor receptor superfamily (Idzerda et al. (1990) *J. Exp. Med.* 171:861). Very recently, a second IL-4R chain was identified, which is the δ_c chain of the IL-2R (Russell et al. (1993) *Science* 262:1877). IL-4R, like IL-2R, may have several functional forms (Rigley et al. (1991) *Int. Immunol.* 3:197).

Because of the widespread effects of IL-4, it is not surprising that the regulation of IL-4 activity is pivotal in determining the outcome of certain diseases (Scott et al. (1988) *J. Exp. Med.* 168:1675) (Heinzel et al. (1989) *J. Exp. Med.* 169:59) (Yamamura et al. (1991) *Science* 254:277) (Zwingenberger et al. (1991) *Scand. J. Immunol.* 34:243) (Wierenga et al. (1990) *J. Immunol.* 144:465). In murine leishmaniasis (Heinzel et al. (1989) *J. Exp. Med.* 169:59), human leprosy (Yamamura et al. (1991) *Science* 254:277), and human schistosomiasis (Zwingenberger et al. (1991) *Scand. J. Immunol.* 34:243),

the production of IL-4 is associated with chronic infection. Increased production of IL-4 in response to allergens characterizes human atopic responses (Wierenga et al. (1990) *J. Immunol.* 144:465). Studies of the molecular regulation of IL-4 activity have previously focused on the effects of promoters, enhancers, and negative regulatory elements within the IL-4 gene (Henkel et al. (1992) *J. Immunol.* 149:3239) (Li-Weber et al. (1992) *J. Immunol.* 148:1913) (Abe et al. (1992) *Proc. Natl. Acad. Sci. USA* 89:2864) (Li-Weber et al. (1993) *J. Immunol.* 151:1371) (Szabo et al. (1993) *Mol. Cell. Biol.* 13:4793).

SUMMARY OF THE INVENTION

Accordingly, a major object of the present invention is to provide an isolated nucleic acid containing exons 1, 3 and 4 of human IL-4.

Another object of the present invention is to provide an isolated nucleic acid containing exons 1, 3 and 4 of human IL-2.

A further object of the present invention is to provide an expression for the isolated nucleic acids containing exons 1, 3 and 4 of human IL-2 and 4.

A still further object of the present invention is to provide polypeptides resulting from the expression of the isolated nucleic acids containing exons 1, 3 and 4 of human IL-2 and 4.

Yet a further object of the present invention is to provide antibodies to the polypeptides resulting from the expression of the isolated nucleic acids containing exons 1, 3 and 4 of human IL-2 and 4.

Another object of the present invention is to provide a method of regulating the activity of human IL-2 and 4 by administering an amount of the polypeptides resulting from the expression of the isolated nucleic acids containing exons 1, 3 and 4 of human IL-2 and 4, respectively, effective to decrease the biological effects of human IL-2 and 4, respectively.

With the foregoing and other objects, advantages and features of the invention that will become hereinafter apparent, the nature of the invention may be more clearly understood by reference to the following detailed description of the preferred embodiments of the invention and to the appended claims.

BRIEF DESCRIPTION OF THE DRAWINGS

Figure 1 shows the detection of two IL-4 mRNA species. Total cellular RNA was extracted from human peripheral blood mononuclear cells (PBMC) stimulated for 6 hours with the anti-CD3 MAb, OKT3, then subjected to reverse transcriptase-polymerase chain reaction (RT-PCR) using oligonucleotide primers specific for exons 1 and 4 of human IL-2, exons 1 and 4 of human IL-4, and interferon-δ (IFN-γ). IL-2, IL-4, and IFN-γ cRNA

internal standards were co-amplified in the same reaction tubes. The RT-PCR amplification products were subjected to gel electrophoresis in a 6% polyacrylamide gel. The 5′ PCR oligonucleotide primer in each pair was end-labeled with ^{32}P, so that amplification products could be detected on autoradiograms. Lane 1 contains molecular weight markers, lane 2 contains IL-2 amplification products, lane 3 contains IL-4 amplification products, and lane 4 contains IFN-γ amplification products.

Figure 2 shows the digestion of IL-4δ2 DNA with *Pst*I but not *Hinc*II. Total cellular RNA was extracted from human PBMC stimulated for 6 hours with the anti-CD3 MAb, OKT3, then subjected to RT-PCR using oligonucleotide primers specific for exons 1 and 4 of human IL-4. The 5′ PCR oligonucleotide primer was end-labeled with ^{32}P. Aliquots of the RT-PCR mixture were undigested (lane 1), or digested with *Hinc*II (lane 2) or *Pst*I (lane 3), which digest IL-4 exons 2 and 3, respectively. The RT-PCR amplification products were then subjected to gel electrophoresis in a 6% polyacrylamide gel. An autoradiogram of the gel showed that *Hinc*II cleaved the 362 bp IL-4 RT-PCR product, but left the 314 bp IL-4δ2 RT-PCR product undigested. *Pst*I cleaved both IL-4 and IL-4δ2 RT-PCR products.

Figure 3 shows the sequence analysis of IL-4 cDNA and cDNA of IL-4 lacking exon 2 (IL-4δ2). IL-4 and IL-4δ2 RT-PCR amplification products were cloned into the

pCR™II vector and their DNA sequences determined using the dideoxy-mediated chain termination method (41). Sequence analysis of IL-4δ2 cDNA demonstrated the presence of IL-4 exons 1, 3 and 4, with exon I spliced directly to exon 3, in frame. Sequence analysis of IL-4 cDNA isolated, cloned, and sequenced in parallel with IL-4δ2 cDNA demonstrated the expected presence of exons 1, 2, 3 and 4. An autoradiogram of the sequencing gel at the region of the IL-4δ2 exon 1-exon 3 splice junction is shown.

Figure 4 shows RNase protection of IL-4 and IL-4δ2 RNA. A radiolabeled IL-4δ2 probe containing an IL-4 exon 1-exon 3 junction was purified and hybridized to 15-20 μg of denatured total cellular RNA from activated PBMC or yeast tRNA. Unhybridized RNA was digested with RNase TI, and the protected RNA fragments were size separated in a 6% denaturing polyacrylamide gel and subjected to autoradiography. Lane 1 shows molecular weight markers, lane 2 shows the purified IL-4δ2 probe, lane 3 shows protection of total cellular RNA from activated PBMC, and lane 4 shows protection of tRNA as a negative control. The 342 bp band in lane 2 represents protected IL-4δ2 RNA and the faint 279 bp band represents protected IL-4 RNA.

Figure 5 shows expression of IL-4 and IL-4δ2 mRNAs in different ratios in different healthy donors. PBMC from 3 healthy individuals were stimulated with anti-CD3 MAb for 6 hours. Expression of IL-4 and IL-4δ2 mRNAs was

tested with RT-PCR using IL-4 exon 1-and exon 4-specific oligonucleotide primers. The 5′ PCR oligonucleotide primer was end-labeled with ^{32}P, so that amplification products could be detected on autoradiograms. The RT-PCR amplification products were then subjected to gel electrophoresis in a 6% polyacrylamide gel. An autoradiogram of the gel showed that the ratio of IL-4:IL-4δ2 mRNA was approximately 2:1 in individual 1 (lane 1), 1: 1 in individual 2 (lane 2), and to 1:2 in individual 3 (lane 3). Lane 4 contains molecular weight markers, and lane 4 contains the negative control RT-PCR products.

Figure 6 shows the expression of IL-4 and IL-4δ2 mRNAs by human T cell clones. The γ/δ T cell clone GIL and the α/β CD4+ T cell clone CAS were each stimulated for 6 hours with anti-CD3 mAb. Expression of IL-4 and IL-4δ2 mRNAs by each clone was tested with RT-PCR using IL-4 exon 1 and exon 4-specific oligonucleotide primers. RT-PCR products were detected by ethidium bromide staining of agarose gels. Both clone GIL (lane 1) and clone CAS (lane 2) produced IL-4 and IL-4δ2 mRNAs, although at different ratios.

Figure 7 shows the kinetics of the expression of IL-4 and IL-4δ2 mRNAs by activated PBMC. PBMC were stimulated with OKT3 MAb, the RNA extracted at the times indicated. Expression of IL-4 and IL-4δ2 mRNAs by each clone was tested with RT-PCR using IL-4 exon 1-and exon

4-specific oligonucleotide primers. The 5′ PCR oligonucleotide primer was end-labeled with ^{32}P. The RT-PCR amplification products were subjected to gel electrophoresis in a 6% polyacrylamide gel. An autoradiogram of the gel is shown, in which lane 1 = 0 hours, lane 2 = 3 hours, lane 3 = 6 hours, lane 4 = 8 hours, lane 5 = 12 hours, and lane 6 = negative control RT-PCR products.

Figure 8 shows that mice do not produce IL-4δ2 mRNA. Spleen cells from BALB/c mice were stimulated with PMA and ionomycin for 24 hours. RNA was extracted and subjected to RT-PCR using murine IL-4 exon 1- and exon 4-specific primers. Human IL-4 and IL-4δ2 mRNA expression was assayed in parallel from anti-CD3 MAb stimulated PBMC with human IL-4 exon 1 and exon 4-specific primers. The RT-PCR products were subjected to agarose gel electrophoresis and detected with ethidium bromide staining. IL-4, but not IL-4δ2, mRNA expression was observed in the murine spleen cells (lane 2), whereas human PBMC expressed both IL-4 and IL-4δ2 mRNA (lane 2). Lane M contains molecular weight markers.

Figure 9 shows the detection of two IL-2 mRNA species. Total cellular RNA was extracted from human PBMC stimulated for 6 hours with the anti-CD3 MAb, OKT3, then subjected to RT-PCR using oligonucleotide primers specific for exons 1 and 4 of human IL-2. In panel A, the 5′ PCR oligonucleotide primer was end-labeled with

^{32}P, and the RT-PCR amplification products were subjected to gel electrophoresis in a 6% polyacrylamide gel. Two RT-PCR products were identified. In panel B, the RT-PCR products were size separated by polyacrylamide gel electrophoresis, transferred to a nylon membrane by blotting, and hybridized with an IL-2 exon 3-specific probe (first autoradiogram) or an IL-2 exon 2-specific probe (second autoradiogram). Lane M contains molecular weight markers in each gel. Lanes 1 and 3 contain RT-PCR products, and lanes 2 and 4 contain negative control RT-PCR products. Two bands hybridized with the exon 3-specific probe (first autoradiogram), whereas only the larger band hybridized with the exon 2-specific probe (second autoradiogram). In a similar experiment shown in panel C, the RT-PCR products were hybridized with an IL-2 exon 1/exon 3 junction specific probe. Lane M contains molecular weight markers, and lane 2 contains RT-PCR products. Two bands hybridize with this probe, and the relative intensity of the smaller band (IL-2δ2) compared to the larger band (native IL-2) is much greater than is seen in panels A or B.

Figure 10 shows the complete sequence of the IL-4 gene (SEQ ID NO:23) (Arai et al, *J. Immunol.*, Vol. 142, pp. 0274-0282 (1989)). The IL-4δ2 (SEQ ID NO:24) of the present invention contains the sequences encoded by exons 1, 3 and 4, but not 2.

Figure 11 shows the complete sequence of the IL-2 gene (SEQ ID NO:25) (Fujita et al, *Proc. Natl. Acad. Sci.*, Vol. 80, pp. 7437-7441 (1983)). The IL-2δ2 (SEQ ID NO:26) of the present invention contains the sequences encoded by exons 1, 3 and 4, but not 2.

DETAILED DESCRIPTION OF THE PREFERRED EMBODIMENTS OF THE INVENTION

The present invention demonstrates the expression of IL-4δ2, a second mRNA isoform transcribed from the IL-4 gene by alternative splicing. Alternative splicing is an efficient mechanism by which multiple protein isoforms may be generated from a single genetic locus. Protein isoforms generated by this regulatory mechanism may vary in function, cellular localization, or pattern of developmental expression (Smith et al. (1989) *Annu. Rev. Genet.* 23:527). Alternative splicing is used in terminally differentiated cells to reversibly modify protein expression without changing the genetic content of the cells (Smith et al. (1989) *Annu. Rev. Genet.* 23:527).

IL-4δ2 was first observed as an additional RT-PCR amplification product during analysis of cytokine gene expression. Cloning and sequencing of the cDNA demonstrated that IL-4δ2 consists of exons 1, 3 and 4 of the IL-4 gene, but not exon 2. Splicing of exon 1 to exon 3 occurs in IL-4δ2 mRNA without changing the reading

frame; exons 1 and 3 are directly opposed at the splice junction without using splice donor or acceptor sites different from those used by IL-4 mRNA. Other than the omission of exon 2, no other changes in the entire protein encoding region are observed when IL-4δ2 and IL-4 mRNAs are compared. To date, all humans tested express both IL-4 and IL-4δ2 mRNAs. Both IL-4 and IL-4δ2 mRNAs increase with T cell activation, and the ratio of IL-4:IL-4,δ2 mRNA increases. A few healthy humans expressed more IL-4δ2 than IL-4 mRNA on occasion, but this finding was not maintained over time in these same individuals. The present invention also demonstrates that external events can change the ratio of IL-4 to IL-4δ2 mRNA.

The IL-4δ2 of the present invention can be isolated from any human immune cell, preferably peripheral blood mononuclear cells (PBMC) and T cells. The cells obtained from a human donor can be separated from blood and other cells using any method known in the art, preferably by density gradient centrifugation, and preferably using a medium such as, but not limited to, Histopaque.

Cells with the appropriate surface markers, including subsets of T cells, preferably $CD4^+$ α/β T cells and γ/δ T cells, can be isolated using any technique known in the art to separate such cell subsets. A particularly preferable method is using positive selection via specific monoclonal antibodies. Especially preferable monoclonal antibodies include anti-Leu3a

specific for CD4, and δTCS1, specific for Vδ1 - Jδ1 and Vδ1 - Jδ2.

Following binding of the MAb to the cells, the cells can be treated with a second antibody specific for the first antibody, which is either coupled to a separation medium, or which can be coupled to a separation medium via a particular linkage, such as a biotin-avidin linkage. Particularly preferable for the present invention is a sheep - anti-mouse IgG coupled to a support such as Dynabeads M-450 (Dynal).

Once the cells are separated, they are cloned in the presence of mitogens, growth factors and/or feeder cells. Preferable mitogens include but are not limited to phytohemagglutinin (PHA) at a concentration of 1-100 μg/ml, preferably at about 10μg/ml. Preferable growth factors include but are not limited to IL-2, at a concentration of 1-100 U/ml, preferably about 50 U/ml. Preferable feeder cells include but are not limited to allogeneic PBMC, preferably irradiated at 1000-10,000 rad, preferably at about 3,000 rad. The cells may also be treated with supernatant from a hybridoma cell line, preferably OKT3, which may stimulate T cell proliferation.

The cells can be grown in any suitable medium, but RPMI is preferable. The medium is preferably supplemented with s rum, such as human serum, preferably human male AB serum, and/or fetal calf serum (FCS). The

serum content is 3-12%, most preferably 10% total serum. It is particularly preferable to use a combination of human male AB serum and FCS, most preferably a mixture of 5% of each serum.

The cells are then expanded, preferably by bi-weekly stimulation with mitogens, feeder cells and growth factors. The expression of surface markers can be confirmed using flow cytometry, fluorescence activated cell sorters (FACS), immunohistochemistry and the like. Preferably, the cells are treated with FITC-conjugated antibodies using standard techniques.

RNA can be extracted from the cells by any means known in the art, preferably using guanidinium thiocyanate. The RNA can then be reverse transcribed into cDNA using known methods, preferably with M-MLV reverse transcriptase and random hexamer primers.

The cDNA generated by reverse transcription of the RNA can then be amplified for further use. Such amplification schemes include but are not limited to polymerase chain reaction (PCR), ligase chain reaction (LCR) and variants thereof. Conditions for such procedures are well known in the art. The amplification products so generated can then be isolated by any technique known in the art. A particularly preferable method is by separation on an agarose gel and electroelution of the product onto DEAE paper followed by phenol/chloroform extraction.

The amplified isolated DNA can then be ligated into a vector suitable for sequencing, transformed into competent cells, and DNA prepared therefrom. Isolation of such plasmids is by techniques well known in the art. The DNA inserts can then be sequenced using any method known in the art, including Maxam-Gilbert sequencing, or preferably by the dideoxy chain termination reaction of Sanger et al.

The RNA of interest can be identified using any means known in the art, but particularly preferable is an RNA protection assay. According to this method, a radiolabelled probe is made which will bind to the RNA of interest. The radiolabelled probe is incubated with total cellular RNA, and unhybridized RNA is digested using RNase. Upon hybridization of the labelled probe to the RNA of interest, the RNA of interest is protected from the RNase and can be identified by electrophoresis on a polyacrylamide gel, with subsequent autoradiography.

Likewise, the cDNAs prepared can be characterized by Southern blot wherein the DNA of interest is run on an agarose gel, the nucleic acids on the gel are transferred to a nylon or nitrocellulose membrane, and the membrane is hybridized with a probe which will aid in the characterization of the DNA. Particularly preferable for the present invention is a probe which spans the exon/exon junctions of an interleukin. Such probes are then able to identify alternative splice mutants.

The above-described methods are suitable for use in detecting expression in various donors and various cells obtained therefrom. In addition, the kinetics of expression can be analyzed to determine whether splice variants are expressed to the same extent as the wild type polypeptides upon stimulation of cells.

The alternative splice variants of the present invention find use in treating various conditions, exemplified but not limited to (1) allergic reactions, including, but not limited to anaphylactic shock, asthma, and eczema; (2) infectious conditions, including, but not limited to leishmania, and for delaying the clinical transition from human immunodeficiency virus (HIV) antibody positivity to acquired immune deficiency syndrome (AIDS); (3) autoimmune disorders, including but not limited to systemic sclerosis and diabetes; (4) fibrotic diseases, including, but not limited to excessive scar tissue formation, excessive extracellular matrix formation, excessive wound healing, and for treating burns; and (5) disorders involving endothelial cells, as IL-4 has been shown to alter the morphology of such cells. In addition, the splice variants of the present invention may be useful in the treatment of any condition which arises from over-expression of the full-length polypeptides.

The present invention not only amplifies a second band using RT-PCR with IL-4 primers, but also

demonstrates that the second band is related to IL-4 using an independent method, an RNase protection assay. The present invention also provides sequence data for the entire protein encoding region to definitively show that the molecule is identical to IL-4, except for the omission of exon 2.

The sequence data disclosed herein show that IL-4 exon 2 functions as a cassette exon (Smith et al. (1989) *Annu. Rev. Genet.* 23:527), and that no shift in the reading frame occurs when it is omitted. The RNase protection assay demonstrates that the IL-4δ2 transcript is expressed in the same sense orientation as IL-4 transcripts, because an anti-sense probe was used for protection.

Also determined was whether the alternative splicing of exon 2 was unique to IL-4 mRNA or part of a more general regulatory mechanism for cytokines. The cytokines tested were IL-2, -3, -5, and GM-CSF, which share protein folding motifs, genomic organization, and receptor extracellular binding domains with IL-4 (Boulay et al. (1992) *J. Biol. Chem.* 267:20525). The present invention also demonstrates that IL-2, but not IL-3, IL-5, or GM-CSF, also uses alternative splicing of exon 2. Both IL-2 and IL-4 splice variants omit exon 2, which encode similar regions of secondary structure and participate in receptor binding for each molecule.

Alternative splicing can be used in humans to provide variants of IL-4 and IL-2 which function as agonists or antagonists of the native cytokines, depending upon the numbers and types of receptors on the cells. By analogy to IL-2 molecules with defined amino acid substitutions (57), IL-2δ2 will still bind to the intermediate affinity IL-2R (β/δ chains) and generate a cellular response. Where loss of the ability to bind to the α chain reduces the capacity of IL-2δ2 to activate cells through the high affinity trimolecular α/βδ complex, the cause is either ineffective triggering or reduction of the assembly of the complex. In these cases, IL-2δ2 is a competitive inhibitor of IL-2 activation through high affinity IL-2R. Similarly, IL-4R has at least two forms with lower (the conventional IL-4R chain alone) and higher (the conventional IL-4R chain plus δc) affinities (Russell et al. (1993) *Science* 262:1877) (Kondo et al. (1993) *Science* 262:1874). IL-4δ2 will bind to the conventional IL-4R chain and serve as an agonist through the lower affinity IL-4R, yet will antagonize cellular activation through the high affinity IL-4R by blocking heterodimerization of the conventional IL-4R chain andδc.

A second species of IL-4 mRNA can be identified using both the reverse transcriptase polymerase chain reaction and an RNase protection assay. This novel IL-4 mRNA is 48 base pairs smaller than IL-4 mRNA, which is

the size of IL-4 exon 2. Sequence data of cloned cDNA demonstrates that this variant contains IL-4 exons 1, 3 and 4, with exon 1 spliced directly to exon 3 in an open reading frame. The entire protein encoding region of this variant, named IL-4δ2, is identical to IL-4, except for the omission of exon 2. IL-4δ2 mRNA is detected in all human PBMC and T cell clones tested, but is absent from mouse spleen cells. Amounts of both IL-4 and IL-4δ2 mRNAs increase upon T cell activation, although IL-4 mRNA increases to a greater extent than does IL-4δ2 mRNA. Similar experiments suggest that humans also express a variant of IL-2 mRNA, in which exon 2 is deleted by alternative splicing. Human IL-3, IL-5, and GM-CSF do not use alternative splicing to delete exon 2. Thus, variants of both human IL-4 and IL-2 exist in which similar structural regions of each molecule are omitted by alternative splicing of mRNA.

The following examples are presented in order to more fully illustrate the preferred embodiments of the invention. They should in no way be construed, however, as limiting the broad scope of the invention.

EXAMPLE 1

Cell Separation and T Cell Cloning.

Human PBMC were isolated from healthy donors by density gradient centrifugation using Histopaque 1077 (Sigma Chemical Co., St Louis, MO). A CD4+ α/β T c ll

clone, CaS, and a γ/δ T cell clone, GIL, were isolated from human PBMC through positive selection using MAb anti-Leu 3a (Becton Dickinson, Mountain View, CA), specific for CD4, and MAb δTCS1 (T Cell Sciences, Cambridge, MA), specific for Vδ1-Jδ1- (36) and Vδ1-Jδ2- (Konig et al. (1989) *Eur. J. Immunol.* 19:2099) encoded epitopes. Subsequent treatment with sheep anti-mouse IgG coupled to Dynabeads M-450 (Dynal Inc., Great Neck, NY) and magnetic bead separation were carried out according to the manufacturer's instructions.

Positively selected cells were immediately cloned by limiting dilution in the presence of 10 μg/ml PHA (Sigma Chemical Co.), 50 U/ml r human IL-2 (Hoffmann-La Roche Inc., Nutley, NJ), and irradiated (3000 rad) allogeneic PBMC as feeder cells. Complete tissue medium was RPMI-1640 containing 5% heat-inactivated human male AB serum, 5% heat-inactivated FCS, 10 mM Hepes, pH 7.4, 2 mM L-glutamine, 1 mM sodium pyruvate, 0.1 mM non-essential amino acid mix, 5 x 10^{-5} M 2-ME, and 5 μg/ml gentamicin sulfate. The T cell clones were expanded in 2 ml cultures by biweekly stimulation with PHA and additional feeder cells. Additional r human IL-2 at the same concentration was added every 4 d. Expression of CD4 and Vδ1 by T cell clones CAS and GIL, respectively, was confirmed using two-color flow cytometric analysis with FITC-conjugated Leu 3a MAb or FITC-conjugated δTCS1 MAb

and PE-conjugated anti-human Leu-4 (CD3) MAb (Becton Dickinson), using standard techniques.

EXAMPLE 2

T Cell Stimulation.

PBMC (5×10^6) or 5×10^6 cloned T cells plus 2.5×10^6 irradiated (3000 rad) allogeneic PBMC were stimulated in 2 ml cultures in complete tissue culture media supplemented to a final concentration of 10% with supernatant of the anti-CD3 MAb secreting hybridoma, OKT3 (American Type Culture Collection, Rockville, MD). This concentration of OKT3 supernatant had previously been determined to optimally stimulate T cell proliferation.

EXAMPLE 3

RNA Isolation and RT-PCR.

Total cellular RNA was isolated from PBMC, T cell clones, and BALB/c spleen cells by acid guanidinium thiocyanate-phenol chloroform extraction (Chomczynski et al. (1987) *Anal. Biochem.* 162:156). One μg of RNA was denatured for 5 minutes at 65°C and then reverse transcribed into cDNA using in a 15 μl reaction mixture containing 200 U of M-MLV reverse transcriptase [Bethesda Research Labs (BRL), Bethesda, MD], 50 mM Tris-HCl, pH 8.3, 75 mM KCl, 8 mM DTT, 3 mM $MgCl_2$, 0.5 mM each dATP, dCTP, dGTP, dTTP (Pharmacia LKB Biotechnology, Piscataway, NY), 1 U/ml RNasin (Promega, Madison, WI),

and random hexamer primers (BRL). This reaction mixture was incubated at 37°C for 1 hour.

A 25 μl PCR reaction mixture was made containing 2.5 μl cDNA mixture, 50 mM Tris-HCl, pH 8.8, 50 mM KCl, 4 mM $MgCl_2$, 0.2 mM each dATP, dCTP, dGTP, dTTP, 0.4 mM each 3′ and 5′ PCR oligonucleotide primers, and 0.625 U Taq polymerase (Perkin Elmer Cetus, Norwalk, CT). The 5′ PCR oligonucleotide primers were 5′ end-labeled with [γ-^{32}P]-ATP (Amersham Corporation, Arlington Heights, IL) and T4 polynucleotide kinase [United States Biochemical (USB), Cleveland, OH], following the USB protocol. The PCR mixture was amplified as follows: denaturation at 95°C for 30 seconds, primer annealing at 60°C for 2 minutes, and primer extension at 72°C for 3 minutes (15-30 cycles), followed with a final 7 minute 72°C extension. Ten PCR products were subjected to gel electrophoresis though 2.5% agarose or 6% polyacrylamide gels. Products of a mock reverse transcriptase reaction, in which H_2O was added in place of RNA, were used as negative control amplifications in all experiments.

The PCR oligonucleotide primer pairs used in these experiments were: human IL-2 exon 1 forward 5′-ATGTACAGGATGCAACTCCTGTCTT-3′ [SEQ ID NO: 1] and exon 4 reverse 5′GTTAGTGTTGAGATGATGCTTTGAC-3′ [SEQ ID NO: 2] ; human IL-3 exon 1 forward 5′ TCCTGCTCCAACTCCTGG-3′ [SEQ ID NO: 3] and exon 4 reverse 5′-GCTCAAAGTCGTCTGTTG-3′ [SEQ ID NO: 4]; human IL-4 pair A exon 1 f rward

5'-TCTTCCTGCTAGCATGTGC-3' [SEQ ID NO: 5] and exon 4 reverse 5'-CGTACTCTGGTTGGCTTTCC-3' [SEQ ID NO: 6]; human IL-4 pair B exon 1 forward 5'-AAGCTTATGGGTCTCACCTCCCAAC-3' [SEQ ID NO: 7] and exon 4 reverse 5'-GGATCCTCATCAGCTCGAACACTTTGA-3' [SEQ ID NO: 8]; murine IL-4 exon 1 forward 5'-AGCCATATCCACGGATGCGAC-3' [SEQ ID NO: 9] and exon 4 reverse 5'-CTCAGTACTACGAGTAATCCAT- 3' [SEQ ID NO: 10]; human IL-5 exon 1 forward 5'-CTTTTTGCAAAAGCCTTGGCCTCCAAAAAAGC-3' [SEQ ID NO: 11] and exon 4 reverse 5'-CCATTCTCCGCCCCAAGGCTGACTAATTTTT-3' [SEQ ID NO: 12]; human GM-CSF exon 1 forward 5'-ATGTGGCTGCAGAGCCTGCTGCTC-3' [SEQ ID NO: 13] and exon 4 reverse 5'TCACTCCTGGACTGGCTCCCAGCA-3' [SEQ ID NO: 14]; and human IFN-γ forward 5'CAGCTCTGCATCGTTTTGGGTTCT-3' [SEQ ID NO: 15] and reverse 5'-TGCTCTTCGACCTTGAAACAGCAT-3' [SEQ ID NO: 16]. *BamHI* and *Hind*III restriction enzyme recognition sequences are underlined in the human IL-4 pair B primers. Construction of the IL-2, IL-4 and IFN-γ cRNA internal standards are described in (Alms, W.J. et al. which is hereby incorporated by reference in its entirety).

EXAMPLE 4

Cloning of RT-PCR Products and DNA Sequencing.

Complementary DNAs for IL-4 and IL-4δ2 were generated and amplified by RT-PCR using IL-4 exon 1 and 4 specific primers containing digestion sites for *BamHI* and

HindIII restriction endonucleases. Amplification products for IL-4 and IL-4δ2 were isolated from 2.5% agarose gels using DEAE paper (Sambrook, J. et al. (1989) Molecular cloning: a laboratory manual Cold Spring Harbor Laboratory Press, New York) (incorporated herein by reference in its entirety). After two phenol/chloroform extractions, the cDNA products were ligated into the pCR™ II vector (Invitrogen Corp., San Diego, CA) and then used to transform INVαF′ competent cells, according to the manufacturer's instructions. Plasmids containing IL-4 and IL-4δ2 cDNA inserts were isolated by conventional techniques (Sambrook, J. et al. (1989) Molecular cloning: a laboratory manual Cold Spring Harbor Laboratory Press, New York) (incorporated herein by reference in its entirety) and used in sequence analyses. IL-4δ2 cDNA inserts were sequenced by the dideoxy-mediated chain termination method (Sanger et al. (1977) *Proc. Natl. Acad. Sci. USA* 74:5463) (incorporated herein by reference in its entirety), using the M13 (-20) forward primer (5′-GTAAAACGACGGCCAGT-3′) [SEQ ID NO: 17] and Sequenase™ (USB), and analyzed by electrophoresis in a 7% Long Range™ (AT Biochem, Malvern, PA) gel. IL-4 and IL-4δ2 cDNA inserts without *Taq* polymerase-induced sequence errors were then used for RNase protection assays.

EXAMPLE 5

RNase Protection Assays.

A 362 bp IL-4δ2 RT-PCR fragment that spanned IL-4 exon 1 to exon 4 with an exon 1-3 junction was cloned into the pCR™ II vector. The insert orientation was determined by sequence analysis. An RNase protection assay was performed using Ambion RPA II™ (Ambion Inc., Austin, TX), according to the manufacturer's protocol. Briefly, radiolabeled IL-4δ2 probe was generated by incubating 100 ng of *SpeI* linearized IL-4δ2-containing plasmid with 5 units T7 RNA polymerase (BRL), 0.5 mM each ATP, CTP, and GTP, 12 μM UTP and 6 μM 400 Ci/mmol 5'[α-^{32}P]-UTP (Dupont NEN, Boston, MA) for 45 min at 37°C. The final specific activity of the IL-4δ2 probe was 1 x 10^9 cpm/μg DNA. The radiolabeled probe was subjected to gel electrophoresis in a 6% denaturing polyacrylamide gel, and the full length IL-4δ2 probe was identified by autoradiography. The band containing the probe was excised from the gel, and the IL-4δ2 probe was eluted at 37°C in 400 μl buffer containing 2 M ammonium acetate, 1% SDS and 25 μg/ml yeast transfer RNA (tRNA). The radiolabeled IL-4δ2 probe (1 x 10^6 cpm) was hybridized with 15-20 μg of denatured total cellular RNA or tRNA for 16 hours at 37°C in 80% formamide, 40 mM PIPES, pH 6.4, 400 mM NaCl, and 1 mM EDTA buffer. Unhybridized RNA was digested at 30°C for 30 minutes with 200 μl RNase

digestion buffer (Ambion Inc.) containing 4000 U/ml RNase T1 (BRL). RNases were inactivated, and the protected RNA fragments were size separated in a 6% denaturing polyacrylamide gel and subjected to autoradiography.

The RNase protection analysis was used to verify the presence of IL-4δ2 mRNA in human PBMC. A 464 bp IL-4δ2 probe containing IL-4 exons 1, 3, and 4, including the exon 1-exon 3 splice junction, was radiolabeled. This probe would be expected to hybridize with and protect a 342 bp fragment of IL-4δ2 mRNA [nucleotides +136 to +198 of exon 1 plus nucleotides +247 to +525 of exons 3 and 4]. In addition, the probe should protect a 63 bp fragment of exon 1 [nucleotide +136 to +198] of IL-4 mRNA and a 279 bp fragment of exons 3 and 4 (nucleotides +247 to +525] of IL-4 mRNA, because IL-4δ2 and IL-4 share these exons. RNase protection of total cellular RNA from anti-CD3 stimulated PBMC verified the presence of both IL-4δ2 (342 bp) and IL-4 (279 bp and 63 bp) fragments (Fig. 4).

EXAMPLE 6

Oligonucleotide Hybridization.

RT-PCR amplification products were size separated by agarose gel electrophoresis. The gel was soaked sequentially for 30 minutes each in denaturation solution (1.5 M NaCl, 0.5 M NaOH) and neutralization solution (1.5 M NaCl, 1 M Tris-HCl, pH 7.4) for 30 minutes. The RT-PCR

amplification products were next transferred to nylon membranes by blotting overnight in 20x SSC buffer. The DNA samples were cross-linked to the membrane by UV light irradiation. Membranes were prehybridized in 6x SSC, 10x Denhardt's solution, 0.1% SDS and 50 μg/ml sperm DNA for at least 1 hour at 42°C and then hybridized overnight with 0.2 μg ^{32}P 5′ end-labeled oligonucleotide probe at 49°C in 6x SSC and 1% SDS. The membrane was washed three times in 6x SSC and 1% SDS for 10 minutes at room temperature, followed by a final 49°C wash. Membranes were then subjected to PhosphorImager analysis (Molecular Dynamics, Sunnyvale, CA) or subjected to autoradiography. Cytokine specific oligonucleotide probe sequences were: human IL-2 exon 2-specific 5′-CTCACCAGGATGCTCACA-3′ [SEQ ID NO: 18]; human IL-2 exon 3-specific 5′-CCTCTGGAGGAAGTGCTA-3′ [SEQ ID NO: 19]; human IL-3 exon 1/exon 3 junction-specific 5′-CCTTTGCTGGAAAATAACC-3′ [SEQ ID NO: 20]; human IL-5 exon 1/exon 3 junction-specific 5′-GCCAATGAGCACCAACTG-3′ [SEQ ID NO: 21]; and human GM-CSF exon 1/exon 3 junction-specific 5′-GCTGAGATGGAGCCGACC-3′ [SEQ ID NO: 22].

Two IL-4 mRNA species were consistently detected from all donors tested (Fig. 1). The larger IL-4 RT-PCR amplification product was 362 bp, corresponding to the predicted size of IL-4 mRNA. The second, smaller RT-PCR amplification product, designated IL-4δ2, migrated with an apparent size of 314 bp. Changes in the PCR buffer

$MgCl_2$ concentration, primer annealing temperature, and pairs of IL-4 exon 1- and 4-specific PCR primers were unsuccessful in eliminating the smaller RT-PCR product (data not shown).

The consistent expression of the smaller 314 bp fragment when total cellular RNA was subjected to RT-PCR and the lack of a corresponding product when an IL-4 cRNA was similarly subjected to RT-PCR (Fig. 1) suggested that this fragment was a specific RT-PCR amplification product resulting from alternative splicing of the IL-4 gene transcript. The IL-4 gene contains 4 exons and 3 introns (Arai et al. (1989) *J. Immunol.* 142:274). The apparent size difference between the IL-4 mRNA RT-PCR product and the IL-4δ2 RT-PCR product was 48 bp, which is the size of IL-4 exon 2. To test whether the 314 bp IL-4δ2 RT-PCR product did not contain IL-4 exon 2, whereas the larger 362 bp IL-4 RT-PCR product did, both products were digested with *HincII* and *PstI*, which digest IL-4 exons 2 and 3, respectively. *HincII* cleaved the IL-4 RT-PCR product, but left the IL-4δ2 RT-PCR product undigested (Fig. 2). In contrast, *PstI* cleaved both IL-4 and IL-4δ2 RT-PCR products (Fig. 2).

EXAMPLE 7

Sequence Analysis of IL-4δ2.

The IL-4 and IL-4δ2 RT-PCR amplification products were then cloned into the pCR™ II vector and their DNA

sequences determined (Fig. 3). Sequence analysis of IL-4δ2 cDNA demonstrated the presence of IL-4 exons 1, 3 and 4, with exon 1 spliced directly to exon 3. Sequence analysis of IL-4 cDNA isolated, cloned, and sequenced in parallel with IL-4δ2 cDNA demonstrated the expected presence of exons 1, 2, 3 and 4, with a exon 2 to exon 3 in-frame splice junction. Of note, both IL-4 and IL-4δ2 contain gaa residues 5′ at exon 2-exon 3 and exon 1-exon 3 splices, respectively. No other sequence changes were observed throughout the entire protein-encoding region of IL-4δ2.

EXAMPLE 8

IL-4δ2 mRNA Expression in Healthy Humans and in Human T Cell Clones.

IL-4 and IL-4δ2 mRNA expression were analyzed in PBMC from 25 healthy humans. IL-4 and IL-4δ2 mRNA were co-expressed in all donors tested, but varied in relative ratio from individual to individual. Examples of this variability are shown in Fig. 5. In this experiment, PBMC from 3 individuals were stimulated with anti-CD3 MAb for 6 hours. The relative expression of IL-4 to IL-4δ2 mRNA was measured by RT-PCR using conditions under which the PCR products were being exponentially amplified (25 cycles). The ratio of IL-4:IL-4δ2 mRNA varied from approximately 2:1 in individual 1 to 1:2 in individual 3. Individual 2 expressed approximately qual amounts of IL-

4 and IL-4δ2 mRNAs. The expression of greater or equal levels of IL-4 than IL-4δ2 mRNA was the predominant phenotype and was present in 22 of 25 individuals tested, with a range of 16:1 to 1:1. Three individuals, however, expressed greater levels of IL-4δ2 mRNA than IL-4 mRNA, on at least one occasion.

To confirm that T cells were the source of IL-4δ2 mRNA expression among the PBMC, cloned T cells were tested. The α/β CD4+ T cell clone CAS and the α/β T cell clone, GIL, were each stimulated for 6 hours with anti-CD3 MAb. Both cloned T cells produced IL-4 and IL-4δ2 mRNAs (Fig. 6).

EXAMPLE 9

Kinetics of IL-4δ2 Expression.

Experiments were done to determine if stimulation of T cells by an anti-CD3 MAb results in the upregulation of both IL-4 and IL-4δ2 mRNA levels and if IL-4δ2 mRNA is regulated independently of IL-4 mRNA. PBMC were stimulated with OKT3 MAb, and the ratio of IL-4δ2 mRNA to IL-4 mRNA was measured at different times (Fig. 7). Both IL-4δ2 and IL-4 mRNAs were expressed spontaneously in these PBMC, with 3.5 times more IL-4 than IL-4δ2 mRNA in this particular experiment. Both IL-4 and IL-4δ2 mRNAs increased with PBMC activation, but IL-4 mRNA increased more than IL-4δ2 mRNA. At 8 hours, 7 times more IL-4 than IL-4δ2 mRNA was present, but by 12 hours, the ratio

had returned to baseline. At 24 and 48 hours, ratios of IL-4 to IL-4δ2 mRNA remained at the baseline of approximately 4 to 1 (data not shown).

EXAMPLE 10

Absence of IL-4δ2 mRNA in Mice.

The human and murine IL-4 genes are each composed of 4 exons and 3 introns, both with a 48 bp exon 2. To determine whether mice also express an alternatively spliced variant of IL-4 with exon 2 deleted, spleen cells from BALB/c mice were stimulated with PMA and ionomycin for 24 hours. RNA was extracted and subjected to RT-PCR using murine IL-4 exon 1- and exon 4-specific primers. Human IL-4δ2 mRNA expression was assayed in parallel from anti-CD3 MAb stimulated PBMC. IL-4, but not IL-4δ2, mRNA expression was observed in stimulated murine spleen cells, whereas human PBMC expressed both IL-4 and IL-4δ2 mRNA (Fig. 8).

EXAMPLE 11

Alternative Splicing of Exon 2 is Also Observed for Human IL-2 mRNA but not Human IL-3, IL-5 and GM-CSF mRNAs.

Because IL-4 belongs to a multigene family of cytokines, IL-2, IL-3, IL-5, and GM-CSF mRNAs were examined to determine whether alternative splicing is used to produce variants that are missing exon 2. Total RNA isolated from human PBMC stimulated for 6 hours with

the anti-CD3 MAb OKT3 was subjected to RT-PCR amplification using exon 1- and exon 4-specific PCR primers for the cytokines of interest. Two RT-PCR amplification products were identified for IL-2 (Fig. 9A). The larger amplification product was 458 bp, which corresponded to the size of native IL-2 mRNA (Fujita et al. (1983) *Proc. Natl. Acad. Sci. USA* 80:7437). The smaller amplification product was approximately 398 bp, a size consistent with an alternatively spliced variant of IL-2 that omitted exon 2. In contrast to the findings with IL-2, only one RT-PCR amplification product each was identified for IL-3, IL-5, and GM-CSF (data not shown).

To further test for the presence of alternative splice variants involving exon 2, IL-2 RT-PCR products were size separated by gel electrophoresis, transferred to a nylon membrane, and hybridized with IL-2 exon 2- or exon 3-specific oligonucleotide probes. Two IL-2 RT-PCR products hybridized with the IL-2 exon 3-specific oligonucleotide probe (Fig. 9B). In contrast, the smaller 398 bp product did not hybridize with an exon 2-specific oligonucleotide, whereas the larger 458 bp product did. This suggests that the smaller 398 bp product is an alternative splice variant of IL-2 that is missing exon 2. In all experiments, the ratios of IL-2δ2:IL-2 mRNA were much lower than the usual ratios of IL-4δ2:IL-4 mRNA, making IL-2δ2 mRNA difficult to detect. To improve detection of IL-2δ2 mRNA, RT-PCR products were

hybridized with an IL-2 exon 1/exon 3 junctional probe (panel C). Because portions of the probe were homologous to exon 1 or exon 3, native IL-2 cDNA was detected with this probe as a larger 458 bp band on the autoradiogram. However, because this probe contained the exon 1/exon 3 junction, IL-2δ2 mRNA was easily discerned as a smaller 398 bp band.

In similar studies, the RT-PCR products for IL-3, IL-5 and GM-CSF were size separated by gel electrophoresis, transferred to a nylon membrane, and hybridized with oligonucleotide probes encoding an exon 1/exon 3 junctional sequence for IL-3, IL-5 and GM-CSF, respectively. No RT-PCR products hybridized with the IL-3, IL-5 or GM-CSF exon 1/exon 3 specific probes (data not shown).

EXAMPLE 12

Rabbit antisera specific for IL-4δ2 protein

A synthetic 16-mer peptide LNSLTEQKNTTEKETF (SEQ ID NO:27) was made. This peptide is specific for the exon 1-exon 3 junction in IL-4δ2 and is not present in IL-4. This peptide was made multimeric through coupling to MAPs resin. Purified multimeric peptide was used to immunize and boost two rabbits, a total of three injections. The post-immunization, but not preimmunization sera from each rabbit binds the IL-4δ2 synthetic peptide, but not recombinant human IL-4 or IL-2, in Western blots.

EXAMPLE 13

Analysis of supernatants from activated human T cell clones for presence of IL-4δ2 protein.

Supernatants from activated human T cell clones were obtained, and the proteins therein were run on SDS-PAGE. Western blots were performed using the antisera obtained in Example 12 on the proteins separated by SDS-PAGE. IL-4δ2-specific antisera bound to IL-4δ2 found in some, but not all of the supernatants tested.

While the invention has been described and illustrated herein by references to various specific material, procedures and examples, it is understood that the invention is not restricted to the particular material combinations of material, and procedures selected for that purpose. Numerous variations of such details can be implied as will be appreciated by those skilled in the art.

SEQUENCE LISTING

(1) GENERAL INFORMATION:

(i) APPLICANT: Alms, William et al

(ii) TITLE OF INVENTION: HUMAN INTERLEUKIN VARIANTS GENERATED BY ALTERNATIVE SPLICING

(iii) NUMBER OF SEQUENCES: 22

(iv) CORRESPONDENCE ADDRESS:
(A) ADDRESSEE: Burns, Doane, Swecker & Mathis
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(C) CITY: Alexandria
(D) STATE: Virginia
(E) COUNTRY: United States
(F) ZIP: 22313-1404

(v) COMPUTER READABLE FORM:
(A) MEDIUM TYPE: Floppy disk
(B) COMPUTER: IBM PC compatible
(C) OPERATING SYSTEM: PC-DOS/MS-DOS
(D) SOFTWARE: PatentIn Release #1.0, Version #1.25

(vi) CURRENT APPLICATION DATA:
(A) APPLICATION NUMBER: To be assigned
(B) FILING DATE: Even date herewith
(C) CLASSIFICATION:

(viii) ATTORNEY/AGENT INFORMATION:
(A) NAME: Crane-Feury, Sharon E
(B) REGISTRATION NUMBER: 36,113

(ix) TELECOMMUNICATION INFORMATION:
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(2) INFORMATION FOR SEQ ID NO:1:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 25 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:1:

ATGTACAGGA TGCAACTCCT GTCTT 25

(2) INFORMATION FOR SEQ ID NO:2:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 25 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:2:

GTTAGTGTTG AGATGATGCT TTGAC 25

(2) INFORMATION FOR SEQ ID NO:3:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 18 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:3:

TCCTGCTCCA ACTCCTGG 18

(2) INFORMATION FOR SEQ ID NO:4:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 18 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:4:

GCTCAAAGTC GTCTGTTG 18

(2) INFORMATION FOR SEQ ID NO:5:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 19 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:5:

TCTTCCTGCT AGCATGTGC 19

(2) INFORMATION FOR SEQ ID NO:6:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 19 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:6:

CGTACTCTGG TTGGCTTCC 19

(2) INFORMATION FOR SEQ ID NO:7:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 25 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:7:

AAGCTTATGG GTCTCACCTC CCAAC 25

(2) INFORMATION FOR SEQ ID NO:8:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 27 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:8:

GGATCCTCAT CAGCTCGAAC ACTTTGA 27

(2) INFORMATION FOR SEQ ID NO:9:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 21 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:9:

AGCCATATCC ACGGATGCGA C 21

(2) INFORMATION FOR SEQ ID NO:10:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 22 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:10:

CTCAGTACTA CGAGTAATCC AT 22

(2) INFORMATION FOR SEQ ID NO:11:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 32 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:11:

CTTTTTGCAA AAGCCTTGGC CTCCAAAAAA GC 32

(2) INFORMATION FOR SEQ ID NO:12:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 31 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:12:

CCATTCTCCG CCCCAAGGCT GACTAATTTT T 31

(2) INFORMATION FOR SEQ ID NO:13:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 24 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:13:

ATGTGGCTGC AGAGCCTGCT GCTC 24

(2) INFORMATION FOR SEQ ID NO:14:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 24 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:14:

TCACTCCTGG ACTGGCTCCC AGCA 24

(2) INFORMATION FOR SEQ ID NO:15:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 24 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:15:

CAGCTCTGCA TCGTTTTGGG TTCT 24

(2) INFORMATION FOR SEQ ID NO:16:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 24 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:16:

TGCTCTTCGA CCTTGAAACA GCAT 24

(2) INFORMATION FOR SEQ ID NO:17:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 17 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:17:

GTAAAACGAC GGCCAGT 17

(2) INFORMATION FOR SEQ ID NO:18:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 18 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:18:

CTCACCAGGA TGCTCACA 18

(2) INFORMATION FOR SEQ ID NO:19:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 18 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:19:

CCTCTGGAGG AAGTGCTA 18

(2) INFORMATION FOR SEQ ID NO:20:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 19 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:20:

CCTTTGCTGG AAAATAACC 19

(2) INFORMATION FOR SEQ ID NO:21:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 18 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:21:

GCCAATGAGC ACCAACTG 18

(2) INFORMATION FOR SEQ ID NO:22:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 18 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:22:

GCTGAGATGG AGCCGACC 18

WHAT IS CLAIMED IS:

1. An isolated nucleic acid comprising exons 1, 3 and 4 of human interleukin-4.

2. The isolated nucleic acid of Claim 1, wherein the nucleic acid is RNA.

3. The isolated nucleic acid of Claim 1, wherein the nucleic acid is DNA.

4. An expression vector comprising the isolated nucleic acid of Claim 3.

5. A transformed cell comprising the vector of Claim 4.

6. The polypeptide expressed by the expression vector of Claim 4.

7. An antibody directed to the polypeptide of Claim 6.

8. An isolated nucleic acid comprising exons 1, 3 and 4 of human interleukin-2.

9. The isolated nucleic acid of Claim 8, wherein the nucleic acid is RNA.

10. The isolated nucleic acid of Claim 8, wherein the nucleic acid is DNA.

11. An expression vector comprising the isolated nucleic acid of Claim 10.

12. A transformed cell comprising the vector of Claim 11.

13. The polypeptide expressed by the expression vector of Claim 12.

14. An antibody directed to the polypeptide of Claim 13.

15. A method of regulating the activity of interleukin-4, comprising administering to a human an amount of the polypeptide of Claim 6 effective to decrease the biological effects of interleukin-4.

16. A method of regulating the activity of interleukin-2, comprising administering to a human an amount of the polypeptide of Claim 13 effective to decrease the biological effects of interleukin-2.

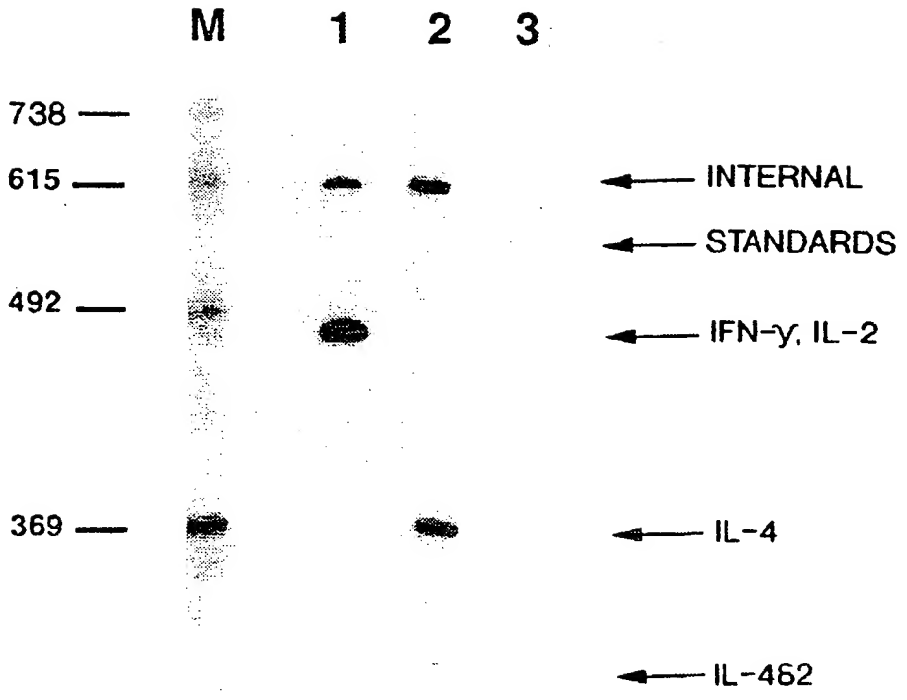

FIG. 1

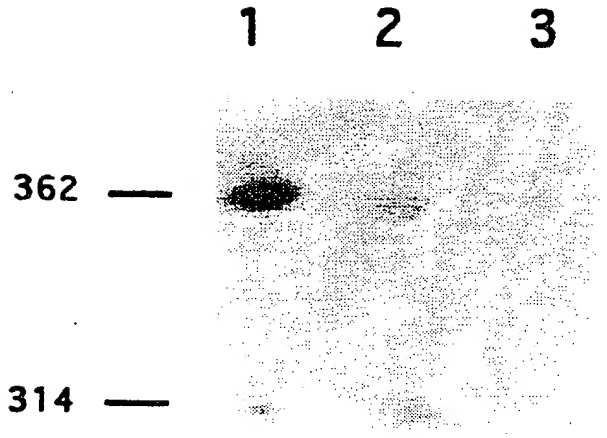

FIG. 2

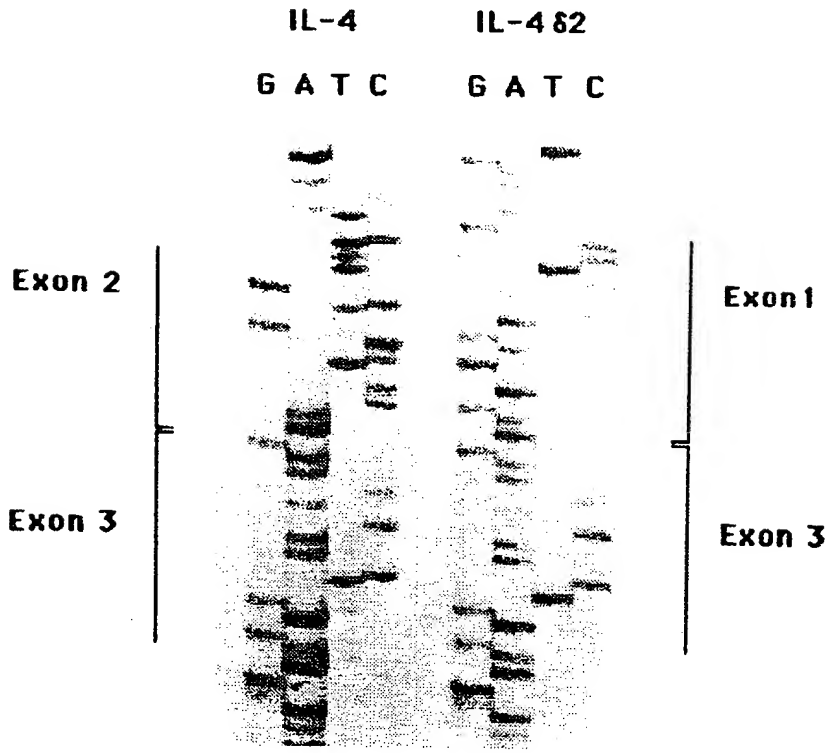

FIG. 3

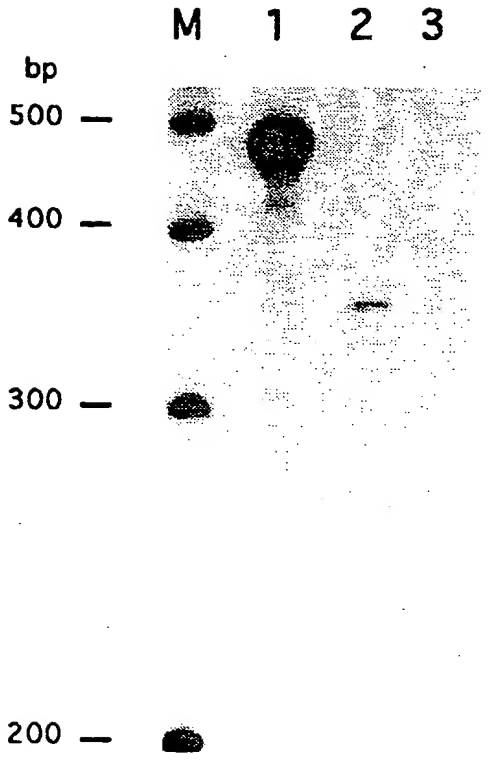

FIG. 4

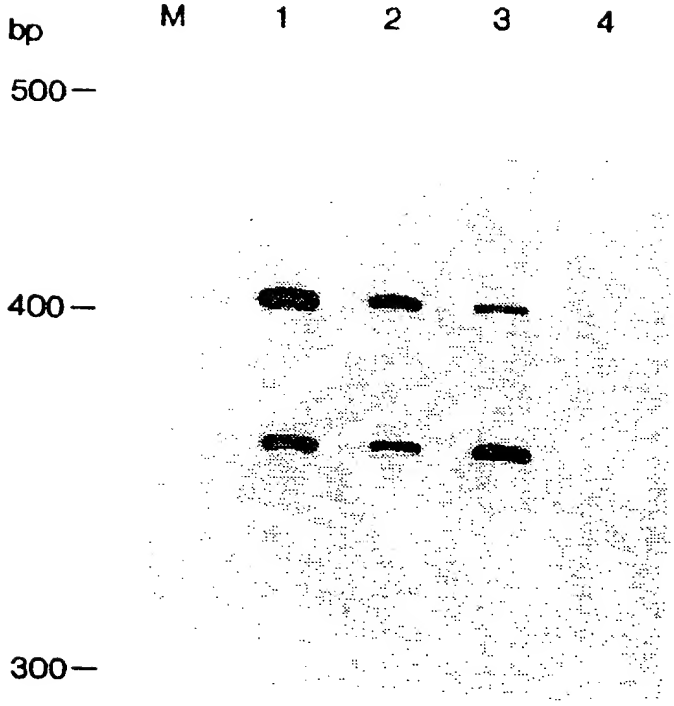

FIG. 5

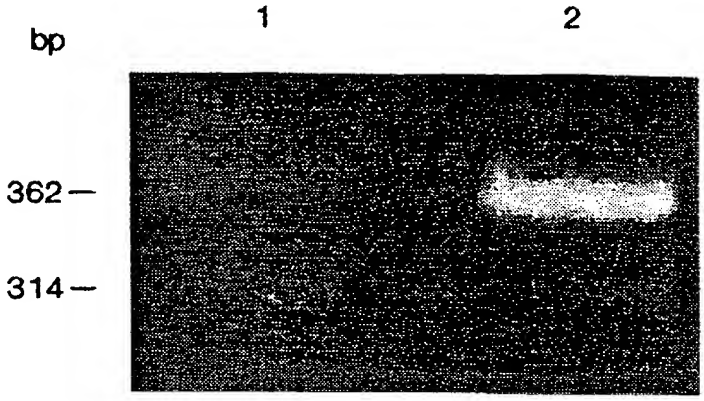

FIG. 6

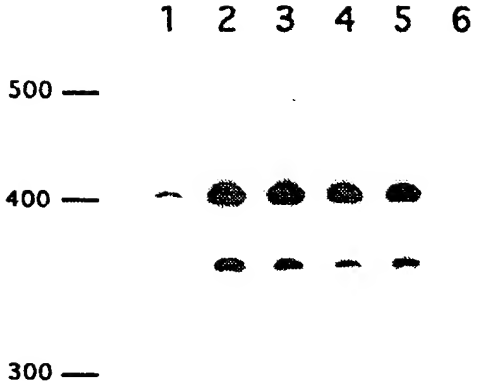

FIG. 7

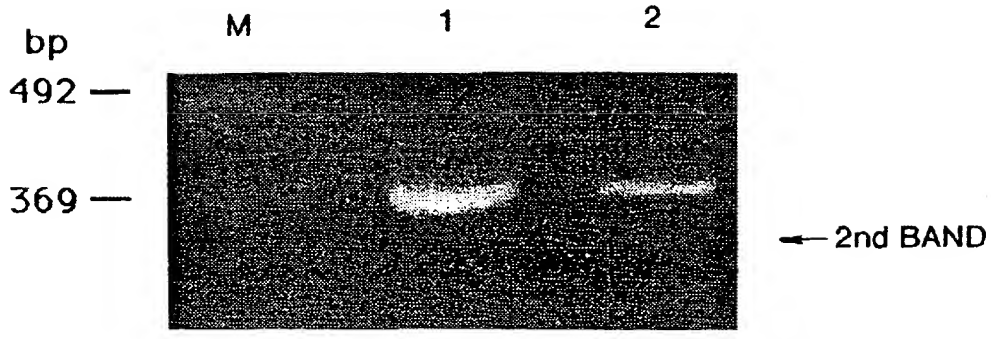

FIG. 8

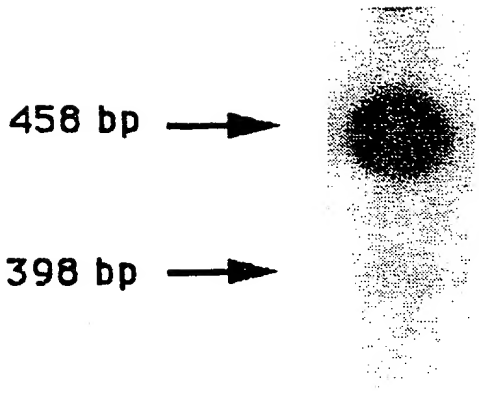

FIG. 9A

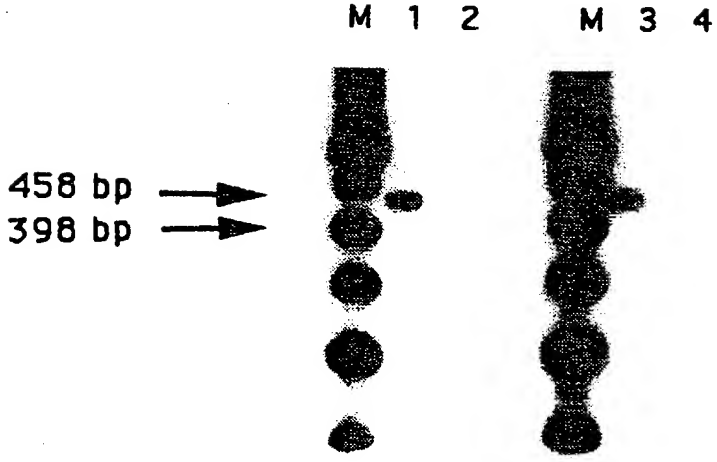

FIG. 9B

FIG. 9C

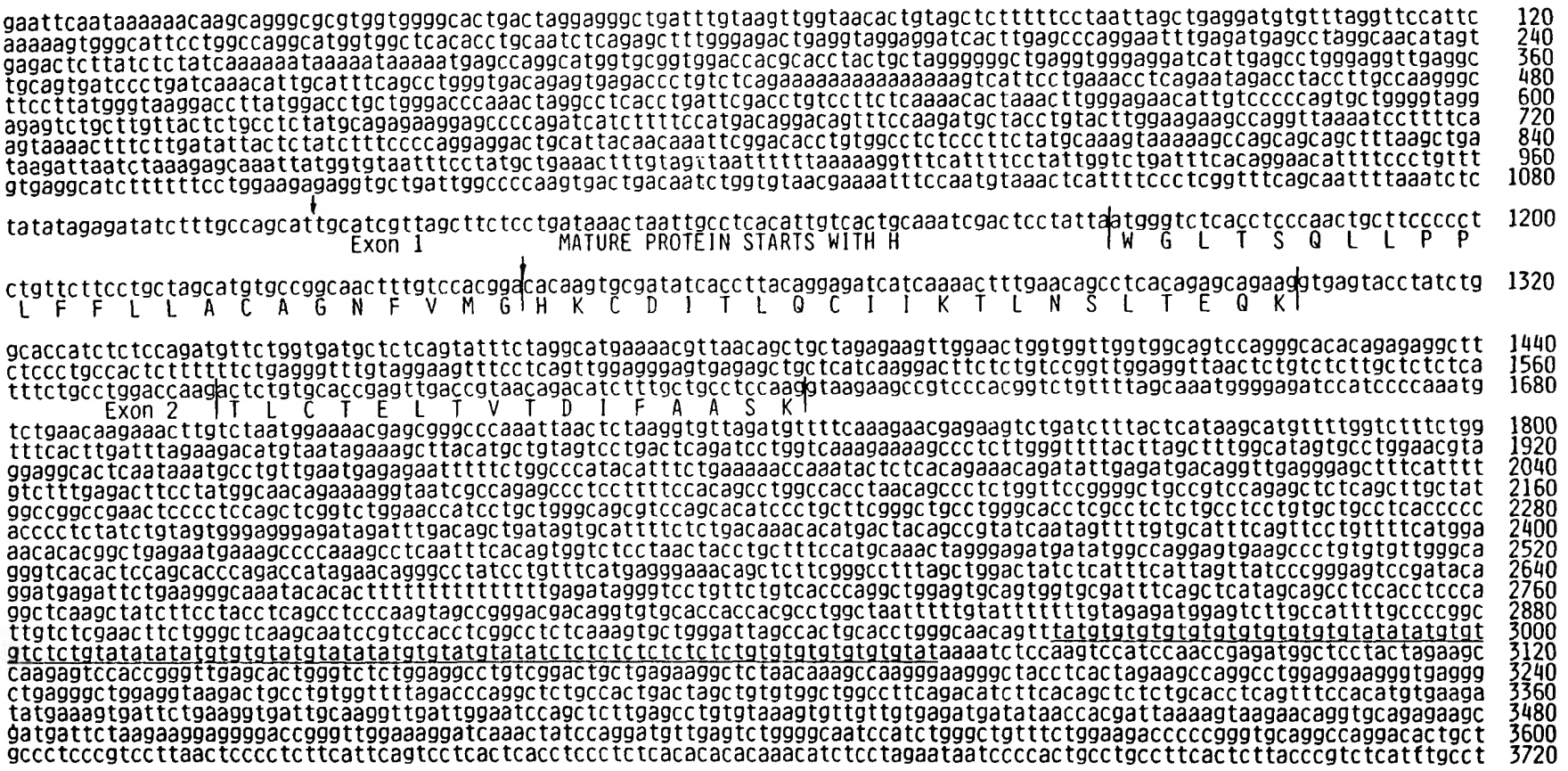

FIG. 10A

```
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FIG. 10B

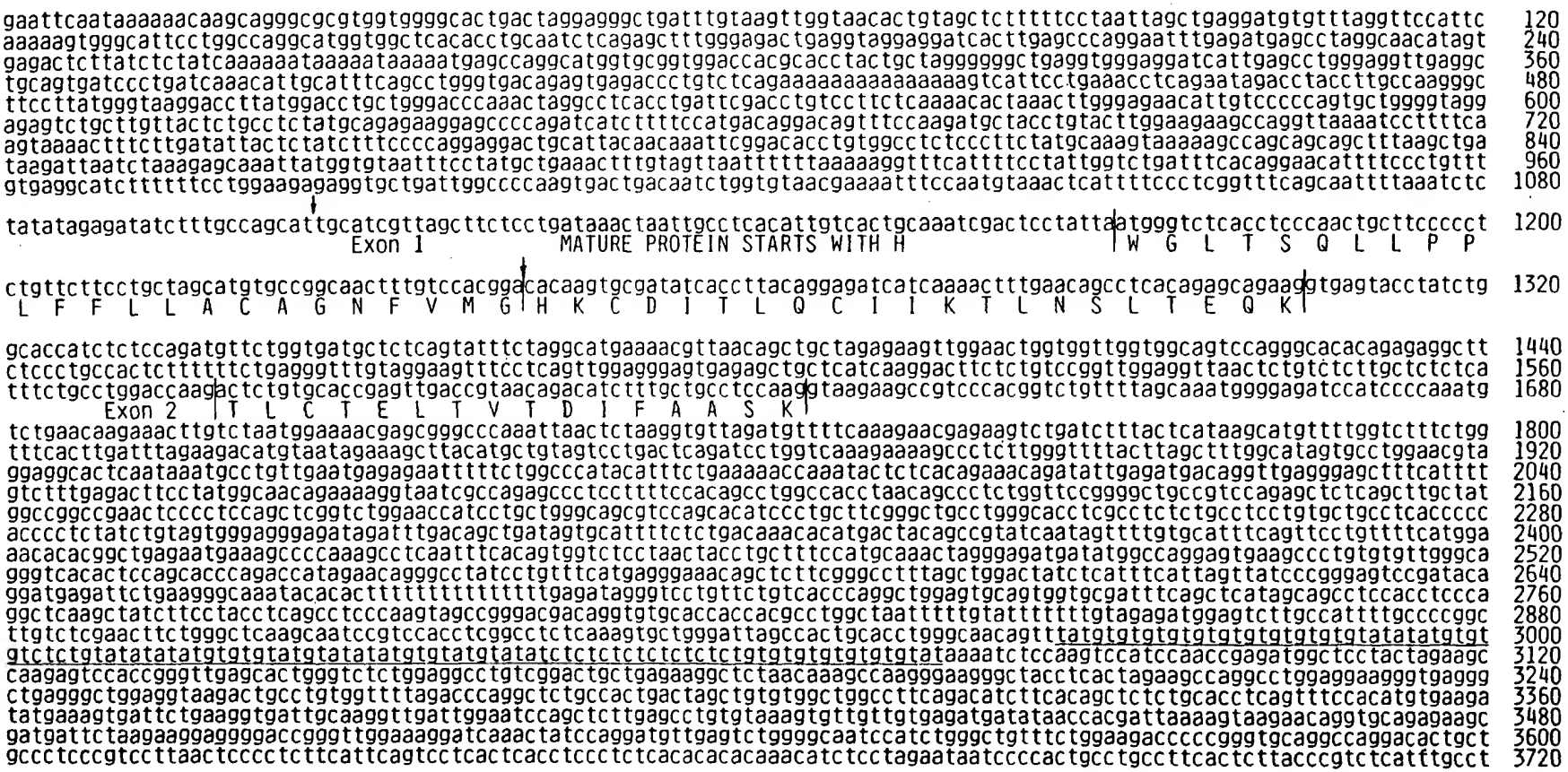


FIG. 11A

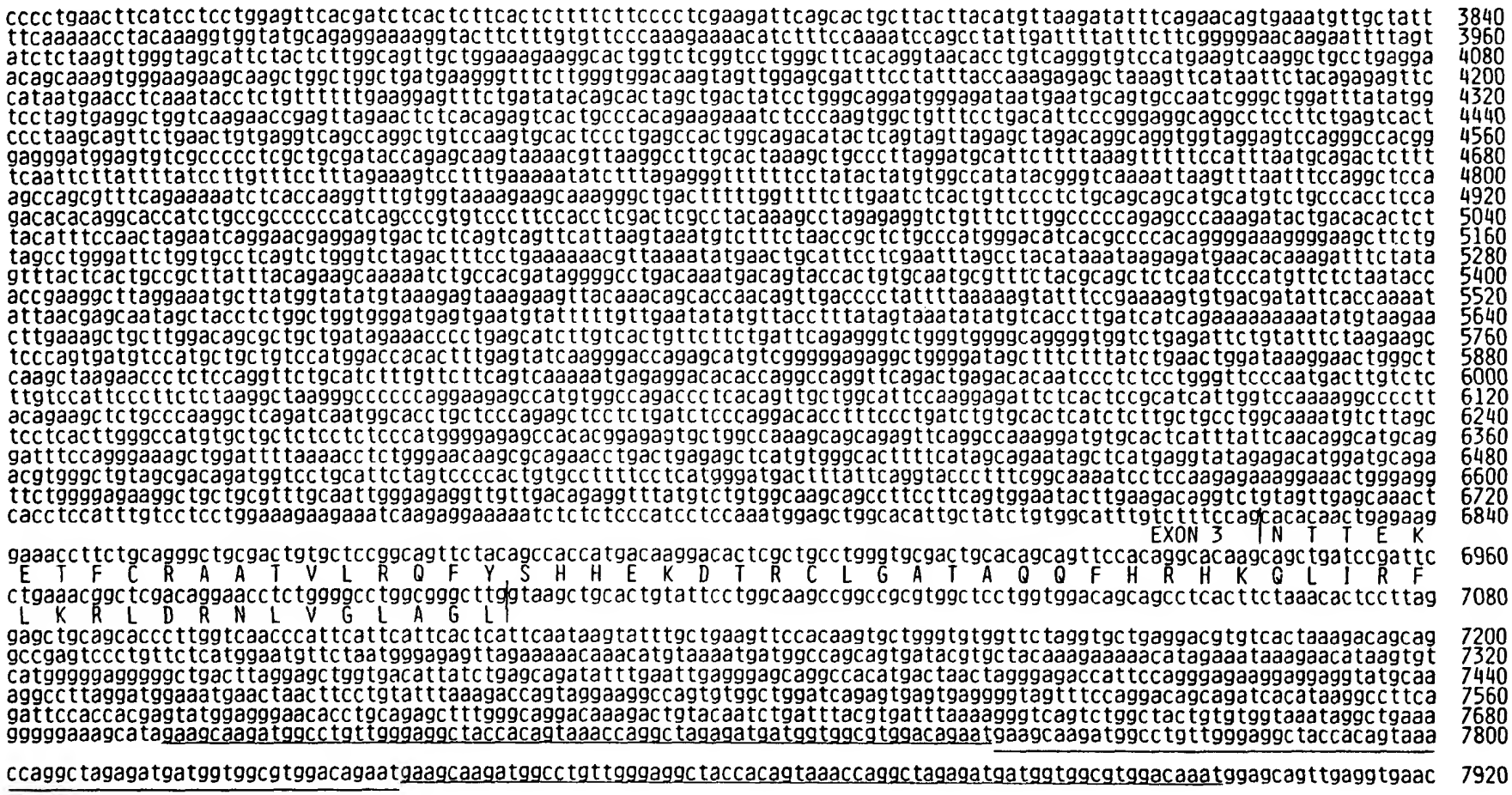


FIG. 11B

SUBSTITUTE SHEET (RULE 26)

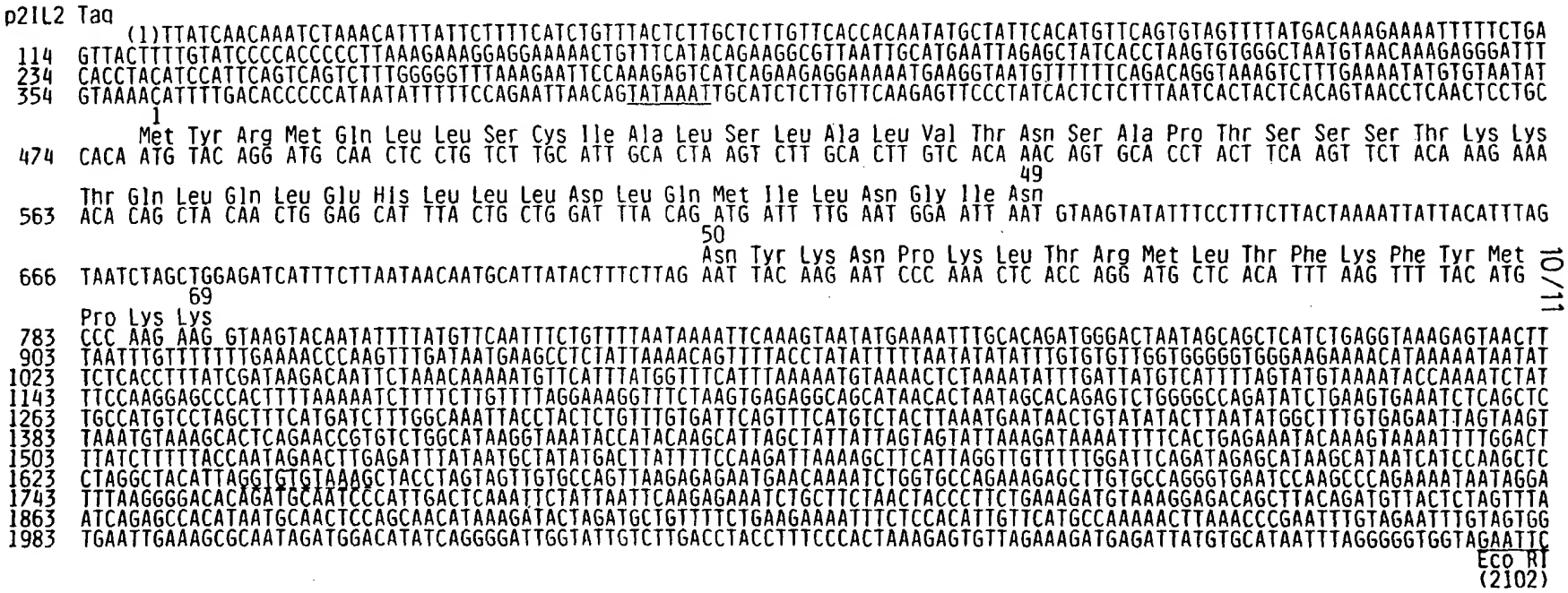
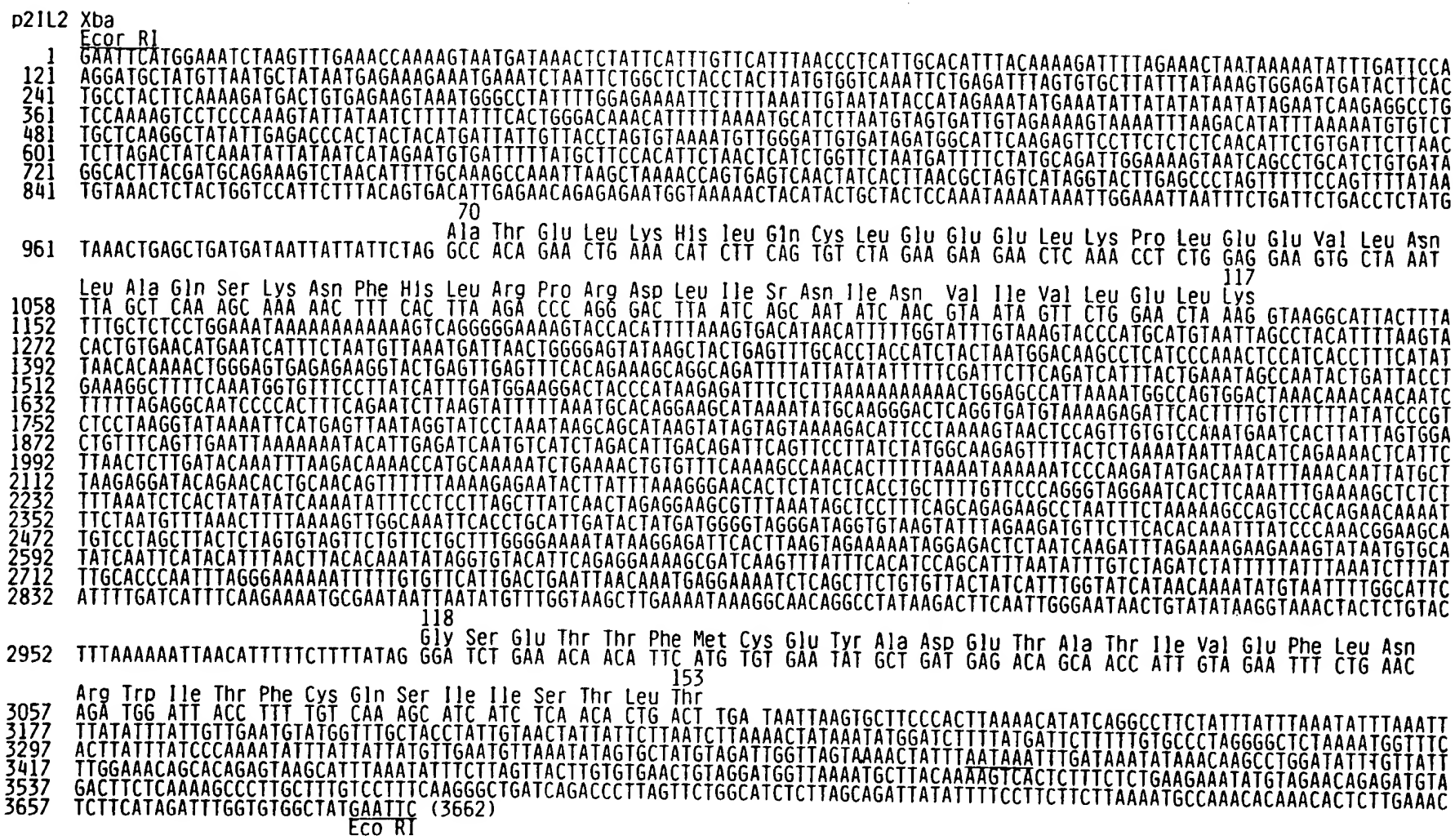


FIG. 12A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Eco RI

FIG. 12B

INTERNATIONAL SEARCH REPORT

I. national application No.
PCT/US95/04094

A. CLASSIFICATION OF SUBJECT MATTER

IPC(6) :C12N 15/00, 1/20; A61K 38/20; C07K 14/52, 16/24

US CL :Please See Extra Sheet.

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

U.S. : 536/23.1; 530/387.1, 351; 424/85.2; 514/1; 435/69.52, 252.3; 930/141

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)

APS, CAS ONLINE, MEDLINE, BIOSIS, SCISEARCH
search terms: interleukin-2, interleukin-4, alternative splicing, variant, antibody, treatment or administration.

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	EXPERIMENTAL HEMATOLOGY, Vol. 21, issued 1993, Sorg et al, "Identification of an Alternatively Spliced Transcript of Human Interleukin-4 Lacking the Sequence Encoded by Exon 2", pages 560-563, see page 560, paragraph 1.	1-3
X, P	IMMUNOGENETICS, Vol. 41, issued 1995, Klein et al, "An Alternatively Spliced Interleukin 4 Form in Lymphoid Cells", page 57, see entire document.	1-3

[X] Further documents are listed in the continuation of Box C. [ ] See patent family annex.

*	Special categories of cited documents:	"T"	later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention
"A"	document defining the general state of the art which is not considered to be of particular relevance	"X"	document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone
"E"	earlier document published on or after the international filing date	"Y"	document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art
"L"	document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)	"&"	document member of the same patent family
"O"	document referring to an oral disclosure, use, exhibition or other means		
"P"	document published prior to the international filing date but later than the priority date claimed		

Date of the actual completion of the international search	Date of mailing of the international search report
09 JUNE 1995	06 JUL 1995
Name and mailing address of the ISA/US Commissioner of Patents and Trademarks Box PCT Washington, D.C. 20231 Facsimile No. (703) 305-3230	Authorized officer PREMA MERTZ Telephone No. (703) 308-0196

Form PCT/ISA/210 (second sheet)(July 1992)*

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US95/04094

C (Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A, P	THE JOURNAL OF CELL BIOLOGY, Vol. 126, Number 5, issued September 1994, LaFlamme et al, "Single Subunit Chimeric Integrins as Mimics and Inhibitors of Endogenous Integrin Functions in Receptor Localization, Cell Spreading and Migration, and Matrix Assembly", pages 1287-1298, see pages 1290-1291.	15-16
A	THE JOURNAL OF IMMUNOLOGY, Vol. 134, Number 6, issued June 1985, Miller et al, "Nucleotide Sequence and Expression of a Mouse Interleukin 2 Receptor cDNA", pages 4212-4217, see abstract.	1-6, 8-13
A	NUCLEIC ACIDS RESEARCH, Vol. 11, Number 13, issued 1983, Devos et al, "Molecular Cloning of Human Interleukin 2 cDNA and Its Expression in *E. coli*", pages 4307-4323, see abstract.	1-14

INTERNATIONAL SEARCH REPORT

I. ..national application No.
PCT/US95/04094

Box I Observations where certain claims were found unsearchable (Continuation of item 1 of first sheet)

This international report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. ☐ Claims Nos.:
because they relate to subject matter not required to be searched by this Authority, namely:

2. ☐ Claims Nos.:
because they relate to parts of the international application that do not comply with the prescribed requirements to such an extent that no meaningful international search can be carried out, specifically:

3. ☐ Claims Nos.:
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

Box II Observations where unity of invention is lacking (Continuation of item 2 of first sheet)

This International Searching Authority found multiple inventions in this international application, as follows:

Please See Extra Sheet.

1. ☒ As all required additional search fees were timely paid by the applicant, this international search report covers all searchable claims.

2. ☐ As all searchable claims could be searched without effort justifying an additional fee, this Authority did not invite payment of any additional fee.

3. ☐ As only some of the required additional search fees were timely paid by the applicant, this international search report covers only those claims for which fees were paid, specifically claims Nos.:

4. ☐ No required additional search fees were timely paid by the applicant. Consequently, this international search report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.:

Remark on Protest

☐ The additional search fees were accompanied by the applicant's protest.

☐ No protest accompanied the payment of additional search fees.

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US95/04094

A. CLASSIFICATION OF SUBJECT MATTER:
US CL :

536/23.1; 530/387.1, 351; 424/85.2; 514/1; 435/69.52, 252.3; 930/141

BOX II. OBSERVATIONS WHERE UNITY OF INVENTION WAS LACKING
This ISA found multiple inventions as follows:

This application contains the following inventions or groups of inventions which are not so linked as to form a single inventive concept under PCT Rule 13.1. In order for all inventions to be examined, the appropriate additional examination fees must be paid.

I. Claims 1-6 and 15, drawn to an isolated nucleic acid of IL-4, an isolated RNA, an isolated DNA, an expression vector, a transformed cell, the IL-4 polypeptide expressed by the expression vector and a method of regulating the activity of IL-4 in a human by administering the polypeptide.

II. Claim 7, drawn to an antibody directed to the IL-4 polypeptide.

III. Claims 8-13 and 16, drawn to an isolated nucleic acid of IL-2, an isolated RNA, an isolated DNA, an expression vector, a transformed cell, the IL-2 polypeptide expressed by the expression vector and a method of regulating the activity of IL-2 in a human by administering the polypeptide.

IV. Claim 14, drawn to an antibody directed to the IL-2 polypeptide.

The inventions listed as Groups I-IV do not relate to a single inventive concept under PCT Rule 13.1 because, under PCT Rule 13.2, they lack the same or corresponding special technical features for the following reasons:

The products/processes of Groups I-IV do not share a special technical feature in that the IL-4 nucleic acid, expression vector, transformed cell, polypeptide expressed and method of regulating the activity of IL-4 of Group I, the IL-4 antibody of Group II, the IL-2 nucleic acid, expression vector, transformed cell, polypeptide expressed and method of regulating the activity of IL-2 of Group III and IL-2 antibody of Group IV do not require each other for their practice and have separate functions all of which constitute the special technical features which define the contribution of each invention. Since these special technical features are not shared by each product and since the common features do not establish an advance over the prior art, the inventions of Groups I-IV do not form a single inventive concept within the meaning of Rule 13.2.